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Drought tolerance in *Folsomia candida* Willem (Collembola) after exposure to sublethal concentrations of three soil-polluting chemicals

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Summary

Drought tolerance (survival) was studied in the collembolan *Folsomia candida* Willem influenced by sublethal concentrations of nonylphenol (40 mg/kg dry soil), linear alkylbenzene sulphonate (LAS) (500 mg/kg) and copper (300 mg/kg). The collembolans were initially exposed to the toxic compounds via soil and thereafter exposed to different degrees of drought stress in the laboratory. *F. candida* survived long-term exposure (7 days) in air with a relative humidity (RH) of 98.5 % at 20 °C. At the lowest humidity (96.8 % RH) survival in control animals was low, between 0 and 30 %. Animals previously exposed to nonylphenol and copper had decreased drought survival compared to control animals, whereas the chemicals alone had no lethal effect. The effects of LAS were not statistically significant although there was a tendency to lowered drought tolerance in exposed animals. The possible physiological mechanisms involved in drought tolerance, and how these may be affected by chemicals, are discussed. The ecological significance of these observations is that severe drought periods may significantly increase the risk of extinction of *F. candida* (or other species) in a polluted habitat compared to an unpolluted habitat.

Key words: Collembola, drought tolerance, toxic chemicals, combined effects, synergism

Introduction

Soil invertebrate populations from time to time encounter environmental bottlenecks such as severe drought in summer or extreme and sudden frosts during autumn and winter. Such climatic stress may reduce populations drastically and ultimately cause local extinction of species. Also soil pollution affects soil invertebrates. Soil pollution may have direct effects on soil invertebrates, e. g. reduced growth and reproduction rates, but it is also likely that xenobiotics reduce the tolerance of climatic stress, or vice versa. Most ecotoxicological risk assessments for soil invertebrates have been based on laboratory tests in which the test organisms have near-optimal conditions with regard to soil moisture and temperature. It is to be ex-

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pected that synergies between physical environmental stress and toxic stress from chemical pollutants will occur in some cases, as, for example, has been demonstrated for marine invertebrates (Depledge 1987; Weber et al. 1992). Although this may seem self-evident, combined effects from natural climatic stress and toxicity of xenobiotic compounds have not been studied in detail for soil invertebrates (Eijsackers and Løkke 1992). Therefore there is a need for research to evaluate the importance of such synergies in order to assess fully the possible effects of xenobiotics on soil invertebrates.

The aim of the present study was to investigate how drought tolerance (survival) in the collembolan *Folsomia candida* Willem is influenced by sublethal doses of three soil polluting compounds. To do this the collembolans were first exposed to the toxic compounds via soil and thereafter exposed to realistic drought stress regimes in the laboratory.

Materials and Methods

Test animals

Test animals were obtained from synchronous cultures of 23–26 days old *Folsomia candida* following Krogh (1995). Before the experiments the animals were held on a moist plaster of Paris/charcoal floor in Petri dishes and fed dried Bakers' yeast. In each experiment (treatment and control) animals from the same batch were used.

Test chemicals and concentrations

Nonylphenol, linear alkylbenzenesulphonate (LAS) and copper were used as test chemicals. These are known to be toxic to collembolans at concentrations found in the environment. Only one sublethal concentration was used. The choice of test concentration for copper was based on preliminary experiments showing that a sublethal effect would be present (e. g. on reproduction) but that mortality would not occur. The test concentration of LAS and nonylphenol was based on data from Holmstrup and Krogh (1996) and Krogh et al. (1996), respectively.

4-nonylphenol (Aldrich) with a mean molar mass of 220.36 g/mol was used in a concentration of 40 mg/kg dry soil. At this concentration reproduction in *F. fimetaria* is negatively affected by about 20 % in the same test soil as used here (Krogh et al. 1996). Nonylphenol was dissolved in 96 % ethanol together with an emulsifier (Tween 20, Merck) before dilution in water. After mixing of the dry soil and test solution the test soil was placed in a fume cupboard overnight to let the ethanol evaporate. After this the water content was adjusted to the desired level and the soil mixed again. As control the same concentration of Tween 20 and ethanol as in treatments was used.

The LAS used was a commercial formulation (MARLON® A 350, 50 % active substance by mass; Hüls Aktiengesellschaft, Marl, Germany) with a mean C-chain length of 11.53 and a mean molar mass of 344 g/mol. A concentration of 500 mg/kg dry soil was used since this has been shown to reduce reproduction in *F. fimetaria* without affecting the mortality of adults (Holmstrup and Krogh 1996). LAS was dissolved in water and mixed into the soil as described by Holmstrup and Krogh (1996). Water was used as control.

Copper was added to the soil as $CuCl_2$ dissolved in water at a concentration of 300 mg Cu/kg. This value was based on preliminary experiments. As control, a NaCl solution with the same concentration of Cl^- as in treated soil was used in order to take into account the possible effects of this ion.

Exposure in soil

The collembolans were exposed to chemicals via soil in a test system described by Holmstrup and Krogh (1996). 30 g moist soil (25.5 g dry soil and 4.5 g demineralized water) and 50 individuals from the synchronous culture (8 replicates in each treatment) were added to each test container. The test soil (LUFA Speyer 2.2, Sp2121; LUFA Speyer, Speyer, Germany) had 2.3 % organic carbon, 5.2 % clay (<0.002 mm), 5.6 % silt (0.002 to 0.02 mm), 34.8 % sand (0.02 to 0.2 mm), 54.6 % gravel (0.2 to 2 mm) and a pH of 5.5. The animals were fed 15 mg dried Bakers' yeast spread on the surface of the soil. After 7 days at 20 °C the soil was spread on a dark surface and the animals were collected by suction and counted. All animals from the 8 replicates were pooled and kept for about one hour on a moist floor of plaster of Paris/charcoal in Petri dishes until they were used in drought experiments.

Drought experiments

After chemical exposure in soil, groups of collembolans were exposed to different drought stresses in the range 99.6 % relative humidity (RH) to 96.8 % RH. This represents a realistic RH regime in soil during periods of natural drought. NaCl solutions (20 ml) covered the bottom of sealed plastic beakers (diameter 7 cm; height 4.2 cm) to control RH of the air in the beakers. The collembolans were placed in a smaller plastic cup, (diameter 1.6 cm; height 3.0 cm) that was floating on the NaCl solution in the beakers. By this method the animals were exposed to air with a precisely defined RH that remained constant during the experiment (Holmstrup and Westh 1995). The NaCl concentrations used and the corresponding RH and water potential are shown in Table 1. For each drought level 4 replicates with 10–14 animals each were set up. After drought exposure for 7 days mortality in the beakers was scored. Animals were scored as dead if there were no movements of antennae or legs when gently touched with a needle.

In a separate experiment the effect of drought exposure on total water content of *F. candida* was estimated by weighing the fresh sample of animals after drought exposure, drying it at 60 °C for 24 hours, and weighing the dry sample by use of a Cahn 4700 automatic electrobalance. The water content was then calculated as (fresh mass-dry mass)/dry mass and expressed as g water/g dry mass.

Table 1. Concentrations of NaCl used in the drought experiments and the resulting relative humidity (RH) in air equilibrated with the salt solutions and the corresponding water potential in pF units

C_s *(g solute/l solution)*	RH *(%)*[1]	pF[2]
7	99.60	3.75
20.2	98.86	4.20
26.4	98.51	4.32
31.6	98.23	4.40
39.0	97.82	4.49
45.3	97.48	4.56
51.7	97.13	4.62
58.1	96.79	4.67

[1] RH of air was calculated from the molar fraction of water in the solution by the formula: $55.56/(55.56 + \text{Osm}) \times 100$ (Edney 1977), where Osm is the osmolality of the solution with a given C_s (data from Weast 1989). One litre of water contains 55.56 moles of water molecules.
[2] Calculated by use of data from Lang (1967)

Results

Survival in contaminated soil

Since the main aim of the study was to investigate how toxic compounds influence drought tolerance, it was necessary to verify that mortality was not due to chemicals alone. Table 2 shows survival rates from the soil exposure experiments. It is seen that survival rates for all chemicals are close to 100 % and that they do not differ from survival rates in control groups. Nor were there any visible differences in activity or general appearance of the animals.

Drought tolerance

In general, survival in *F. candida* was unaffected down to 98.5 % RH (Fig 1). At lower RH, survival was decreasing and at 96.8 % RH (the lowest used) survival was low, with mean values between 0 and 30 %. In the experiment with nonylphenol the exposed animals were significantly less drought tolerant than control animals. In the control, mean survival rates at

the two lowest RHs were 30–50 %, whereas treated animals did not survive at all. Animals that had been exposed to LAS showed a weak but consistent tendency to lower survival rates than controls. This was also the case for animals that had been exposed to copper. Table 3 shows the RH causing 50 % mortality (LRH_{50}) in all experiments. As judged from the estimated 95 % fiducial confidence limits nonylphenol and copper significantly decreased drought survival in *F. candida* in comparison with the corresponding control. The observed decrease in drought survival of animals exposed to LAS was not statistically significant.

Table 2. Mean survival rates (± SEM) of *F. candida* exposed to nonylphenol (40 mg/kg dry soil), LAS (500 mg/kg) or $CuCl_2$ (300 mg Cu/kg) for 7 days in soil. The values are based on 8 replicates with 50 individuals each

Experiment	Survival in controls (%)	Survival in treatments (%)
Nonylphenol	97.8 ± 1.0	98.8 ± 0.6
LAS	97.0 ± 1.1	97.3 ± 0.8
$CuCl_2$	97.3 ± 0.8	97.5 ± 0.7

Table 3. Drought tolerance in *F. candida* expressed as the relative humidity (%) causing 50 % mortality (LRH_{50} values with 95 % fiducial confidence limits) in experiments where animals were previously exposed to sublethal concentrations of nonylphenol (40 mg/kg dry soil), LAS (500 mg/kg) or $CuCl_2$ (300 mg Cu/kg) in soil for 7 days

Experiment	LRH_{50} control (%)	LRH_{50} treatment (%)
Nonylphenol	97.05 (96.92–97.16)	97.76 (97.69–97.84)
LAS	97.32 (96.38–97.60)	97.52 (96.44–99.07)
$CuCl_2$	96.96 (96.82–97.07)	97.24 (97.13–97.33)

Note: LRH_{50} values were calculated with the PROBIT procedure in SAS (SAS Institute, 1988) after omitting data for the three highest RH values

Effects of RH on body water content

Body water content of *F. candida* was highly influenced by RH (Fig. 2). At the highest RH used (99.6 % RH) water content was approximately 3.4 g/g dw, which is about the value generally reported for fully hydrated Collembola (Petersen 1975; Verhoef and Li 1983; Witteveen et al. 1987). With decreasing RH the water content declined to about 1.3 g/g dw at 96.8 % RH. This is 62% lower than the water content at 99.6 % RH. The water content at 98 % RH, below which mortality began to increase, is approximately 1.9 g/g dw. This corresponds to a 44 % reduction of the water content of fully hydrated animals.

Discussion

Folsomia candida belongs to the euedaphic Collembola, i.e. species that are often eyeless and without pigment, living in the soil pore system. It is mostly found in soil rich in organic content such as compost (Gisin 1960). As compared to surface dwelling Collembola and most

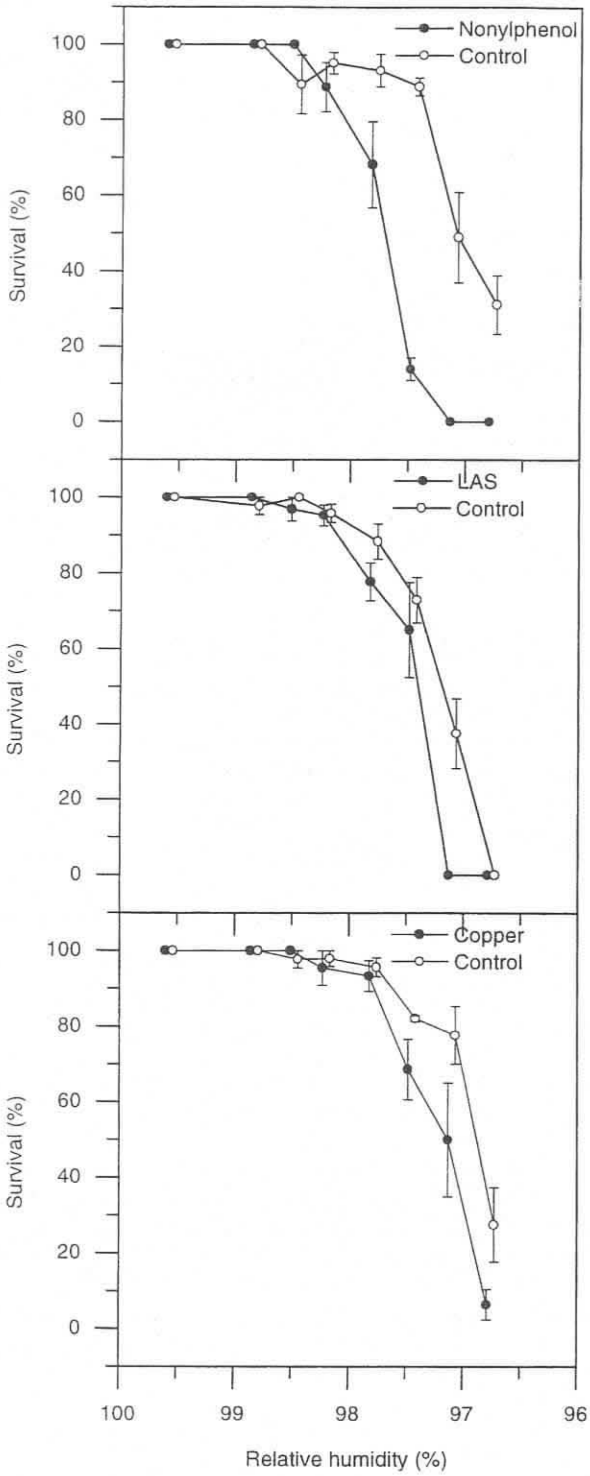

Fig. 1. Mean survival rates (± SEM) of *Folsomia candida* kept at various relative humidities for 7 days at 20 °C. Closed circles represent treated animals, previously exposed to nonylphenol (40 mg/kg dry soil), $CuCl_2$ (300 mg Cu/kg) or LAS (500 mg/kg) for 7 days in soil at 20 °C. Open circles represent control animals, previously kept in control soil for 7 days at 20 °C. Each value is based on 4 replicates with 10 to 14 animals each (for clarity the points of the two treatments have been slightly displaced relative to each other in the X-axis direction). Note that desiccation stress increases from left to right

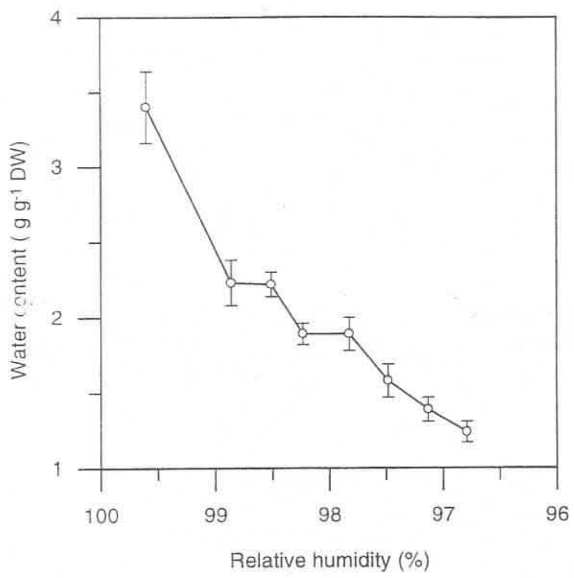

Fig. 2. Mean water content (± SEM) of *Folsomia candida* kept at various relative humidities for 7 days at 20 °C. Each value is based on 4 replicates consisting of 10–14 animals

insects, euedaphic Collembola live in a very buffered habitat with respect to desiccation (Vannier 1983). The air in the pore space of soils is practically saturated with water vapour when the soil appears moist (Hillel 1971). Even at water potentials around the permanent wilting point for plants (pF 4.2), which appears as a very dry soil, RH is as high as 98.9 % (Table 1). Nevertheless, euedaphic Collembola have relatively little resistance to desiccation because they have soft bodies and high rates of cuticular water loss. Hence, exposure to humidities even slightly below saturation may be damaging. This was clearly demonstrated for *F. candida*, which in the control situation had increasing mortality below 98.2 % RH (Fig. 1). These results were obtained from relatively long-term experiments where the water content of the animals had come into equilibrium with the surrounding atmosphere (data not shown). They therefore indicate the levels of desiccation *F. candida* can tolerate for longer periods in its habitat. Apparently this species easily survives soil water potentials around the permanent wilting point of plants. Hemolymph osmolality of summer acclimated Collembola is usually 275 to 350 mOsm/kg (Weigman 1973; Verhoef & Li 1983; Witteveen et al 1987; Zettel 1984). This corresponds to 99.5 to 99.3 % RH (cf. note in Table 1). Below these RH values vapour pressure of air is lower than that of the body fluids, resulting in cuticular water loss. This explains why animals were apparently fully hydrated at 99.6 % RH, but lost significant amounts of water at declining RH (Fig. 2).

It was demonstrated that sublethal concentrations of nonylphenol and copper reduced the drought tolerance in *F. candida*, whereas LAS had a less evident effect. There may be different mechanisms operating in the observed decline of drought tolerance. One possibility is that the lowest tolerable water content of exposed animals is higher than that of unexposed animals. For example, animals exposed to 97.7 % RH had lost about 50 % of their normal water content (Fig. 2). This would cause about a doubling of the concentration of the toxicant dissolved in the body fluids, which may increase effects. Another possibility is that physiological water conserving mechanisms are affected by the toxicant. These questions need to be investigated in further studies, but will be discussed in brief here.

Terrestrial invertebrates have at least two physiological strategies to avoid reaching the lower critical water content: 1) they can to some extent reduce water loss by having an impermeable cuticle and 2) they may accumulate solutes (polyols, salts, amino acids) to minimize the vapour pressure difference between surrounding air and body fluids and with that the tran-

spiration rate (Edney 1977; Zachariassen 1985). Nonylphenol and LAS are surface active compounds (detergents) and as such they may cause disruption of biomembranes and denaturation of proteins (Schwuger and Bartnik, 1980). These effects could lead to increased water permeability of the cuticle of *F. candida* and a reduced capacity to retain body water. Decreasing body size increases the overall water loss rate because of the increased surface-to-volume ratio (Edney 1977). It could be argued that exposed animals had grown to a smaller size than control animals during the seven day exposure period, and therefore would have a greater water loss rate resulting in a lower survival. The size of the animals was not measured systematically, but there were no visible differences between treated and untreated animals. It is therefore believed that the observed differences in survival rate are due to truly physiological effects of the test chemicals.

Even though copper is an essential trace metal it may exert toxic effects if it enters into biochemical reactions in which it is not normally involved (Hopkin 1989). It can therefore be speculated that specific biochemical reactions of importance for drought tolerance are affected by some toxicants. It is not known if LAS and nonylphenol can cause similar disturbances. Another mechanism may be that detoxification and excretion of toxic compounds are probably energetically costly for the animals (Calow 1991). The "scope for maintenance" of the exposed animals may therefore be reduced by the toxicants so that a "natural" stress (here: drought), normally tolerated by the species, now becomes lethal.

In the present study effects of only three chemicals were tested. It is likely that many other xenobiotic compounds will have similar effects to a higher or lesser degree. At the single concentration used in the experiments there was, at least for nonylphenol, a distinct synergy between chemical exposure and drought. The ecological significance of this is, that during severe drought periods the risk of extinction of *F. candida* (and other species) in a polluted habitat may be much higher than in an unpolluted habitat.

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